MS93.P01

Acta Cryst. (2011) A**67,** C768

X-ray structure reveals a new class of alkaline phosphatase

Subhash C. Bihani,[a] Amit Das,[a] Kayzad S. Nilgiriwala,[b] Prashar V.,[a] S.K.Apte,[b] J.L.Ferrer,[c] and M. V. Hosur,[a] [a]*Solid state phyics division,* [b]*Molecular Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai – 400085, (India).* [c]*Groupe Synchrotron, Institut de Biologie Structurale J-P Ebel, CEA-CNRS-UJF, Grenoble, (France).* E-mail:scbihani@barc.gov.in

Alkaline phosphatases (EC 3.1.3.1) hereafter referred to as APs, are metalloenzymes that hydrolyze the phosphate monoesters under alkaline pH conditions. APs are widely distributed in nature from prokaryotes to eukaryotes, and show substantial divergence with respect to their molecular weight, pH and temperature optima, thermal stability, metal requirement, specific activity and cellular location. The AP isolated from the bacterium *Sphingomonas.* sp. Strain BSAR-1 (SPAP), is a novel enzyme with properties very suited for use in molecular biology and bioremediation applications. Here we report the first three dimensional crystal structure of SPAP bound by an organic phosphate monoester. The structure is solved by MAD method using selenomethionine derivative. Native structure is refined to 1.95 Å resolution with R and R_{free} of 15.53 and 18.63 respectively. The structure shows that SPAP is the first AP in which: 1) the catalytic residue is threonine instead of serine and 2) third metal ion binding pocket near the active site observed in all APs so far is abolished in SPAP. When compared to AP from *E. coli* (ECAP) and other ECAP-like APs, the architecture of the active site is similar only to the extent that a bimetallic zinc core is coordinated by conserved His and Asp residues, while the combination of residues binding the substrate phosphoryl group is different. Individual residues of this combination are observed only separately in different members of the AP superfamily.

The conserved arginine residue in the active site of AP from *E. coli* (ECAP) is deleted in SPAP, and a lysine, an arginine and an aspargine residue recruited together for the first time into the active site, bind the substrate phosphoryl group in a manner not observed before in any other AP. The characteristic pair of hydrogen bonds between active site arginine and nonbridging posphoryl oxygens from the substrate is replaced by two non-coplanar hydrogen bonds contributed by Asn and Arg. This mode of interaction may be an alternate way of stabilizing the bi-pyramidal transition state in alkaline phosphatases. A positively charged lysine residue interacts directly with two nonbridging phosphoryl oxygens, and this could lead to enhanced catalysis. These and other structural features suggest that SPAP represents a new class of APs, additional members of which could be discovered in the future. However, the orientation of the phosphoryl moiety in the active site is consistent with the in-line displacement mechanism mediated by a trigonal-bipyramidal transition state proposed for ECAP-like APs. The structure is suggestive of independent evolutionary paths for SPAP and ECAP-like APs, and also of the likelihood that NPPs evolved from SPAP-like structure rather than from ECAP-like structure as suggested earlier. The structure can be used to identify residues that can be modified to engineer SPAP for molecular biology applications.

Keywords: X-ray structure, alkaline phosphatase, MAD

MS93.P02

Acta Cryst. (2011) A**67,** C768

Structural insights into the mechanism of drug activation by azoreductases

Ali Ryan,[a,b] Elise Kaplan,[a] Nicola Laurieri,[a] Edward Lowe,[c] Edith Sim,[a,b] [a]*Departament of Pharmacology, University of Oxford, Oxford, (UK).* [b]*Faculty of science, engineering and computing, Kingston University, Kingston-upon-thames, (UK).* [c]*Laboratory of Molecular Biology, Department of Biochemistry, University of Oxford, Oxford, (UK).* E-mail: ali.ryan@pharm.ox.ac.uk

Drug metabolism in the gut is an important but so far poorly understood part of the process of drug absorption into the body. For some time azoreductases have been known to be vital for the activation of azo prodrugs such as balsalazide in the gut. Azoreductases are NAD(P)H dependent flavoenzymes found in species ranging from bacteria to humans [1]. As well as being important for drug metabolism knockout studies in bacteria suggest azoreductases might be valid targets for novel antibiotics. Azoreductases are able to reduce a chemically diverse range of substrates including azo drugs, quinones, nitroaromatics and metal ions. The mechanism by which they reduce these varied substrates has remained elusive until recently. Three azoreductases from *P. aeruginosa* have been extensively characterized [1], [2]. Recent structural studies have focused on one of these enzymes (paAzoR1) and have significantly improved our understanding of their reaction mechanism. Structural data showing paAzoR1 bound to the inflammatory bowel disease prodrug balsalazide (PDB: 3LT5) have significantly altered our perception of how azoreduction occurs and provide direct evidence for the mechanism of quinone reduction [1]. The proposed quinone reduction mechanism has subsequently been supported by the structures of paAzoR1 bound to Coenzyme Q_1 and anthraquinone-2-sulphonate. Data from paAzoR1 bound to the topical antibiotic nitrofurazone, have provided evidence for the mechanism of nitroaromatic reduction by azoreductases and suggests there may be a conserved mechanism among structurally diverse nitroreductases [3].

[1] A. Ryan, N. Laurieri, I. Westwood, C.J. Wang, E. Lowe, E. Sim, *Journal of Molecular Biology*, **2010**, *400*, 24-37. [2] C.J. Wang, C. Hagemeier, N. Rahman, E. Lowe, M. Noble, M. Coughtrie, E. Sim, I. Westwood, *Journal of Molecular Biology*, **2007** ,*373*, 1213-1228. [3] A. Ryan, E. Kaplan, N. Laurieri, E. Lowe, E. Sim, *Molecular pharmacology*, **2011**, submitted.

Keywords: azoreductase, mechanism, nitroaromatic

MS93.P03

Acta Cryst. (2011) A**67,** C768-C769

First structural evidence for the order of preference of inorganic substrates by lactoperoxidase

Punit Kaur, Nisha Pandey, Amit K. Singh, Mau Sinha, Sujata Sharma, Tej P. Singh, *Department of Biophysics, All India Institute of Medical Sciences, New Delhi – 110 029 (India).* E-mail: kaurpunit@gmail.com

Lactoperoxidase (LPO) catalyzes the oxidation of halides and pseudohalides by hydrogen peroxide to produce a wide range of antimicrobial activity. LPO has been co-crystallized with a mixture containing equimolar concentrations of SCN^-, I^-, Br^- and Cl^-. The structure determination revealed the positions of four linearly arranged halides in the substrate-diffusion channel. The bound substrate ions are separated from each other by one or more water molecules. The heme iron is coordinated to His-351 $N^{\varepsilon 2}$ on the proximal side while it is coordinated to conserved water molecule W-1 on the distal heme side. W-1 is hydrogen bonded to Br^- ion which is followed by Cl^- ion with a hydrogen bonded water molecule W-5′ between them. Next to Cl^- ion is a hydrogen bonded water molecule W-7′ which is hydrogen bonded to W-8′ and N atom of SCN^- ion. W-8′ is hydrogen bonded to W-9′ which in turn is hydrogen bonded to I^- ion. I^- is also hydrogen bonded to Thr-425 N. On the other hand SCN^- interacts directly with Asn-230 and through water molecules with Ser-235 and Phe-254. According to the locations of four substrate anions in the channel with respect to the